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(54) Title: METHOD FOR TREATING SYMPTOMS OF DIABETES

(57) Abstract

The present invention provides a method for treating symptoms of diabetes in a diabetic subject which comprises administering to the subject a therapeutically effective amount of an agent which inhibits binding of advanced glycation endproducts to any receptor for advanced glycation endproducts so as to treat chronic symptoms of diabetes in the subject.

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Method for Treating Symptoms of Diabetes

The invention disclosed herein was made with Government support under Grant Nos. HL21006 and AG00603 from the National Institutes of Health, U.S. Department of Health and Human Services. Accordingly, the U.S. Government has certain rights in this invention. This application claims the priority of U.S. Serial No. 08/755,235, filed November 22, 1996.

Background of the Invention

Throughout this application, various publications are referenced by author and date. Full citations for these publications may be found listed alphabetically at the end of the specification immediately the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein.

Ineffective healing of wounds is a serious problem in diabetes, contributing to increased morbidity (Reynolds, 1985; Galloway and Shuman, 1963; and Pearl and Kanat, 1988). The reparative response in wound healing is orchestrated by multiple cellular elements which work together in many ways, including infiltration of the lesion by inflammatory effector cells. Subsequent to this, fibroblastic elements together with inflammatory effector cells provide antibacterial mechanisms and promote removal of necrotic tissue, as well as laying down of new connective tissue. A fundamental disorder of glucose metabolism might perturb these complex and interactive protective processes. Previous work has suggested that cellular dysfunction in diabetic wound healing involves defective neutrophil function (Bagdade et al., 1978; Nolan et al., 1978; and Mowat and Baum, 1971), delayed infiltration of the wound with inflammatory cells (Greenhalgh et al., 1990 and Fahey

et al., 1991), decreased production of collagen (Goodson and Hunt, 1977 and Goodson and Hunt, 1986), and diminished activity of endogenous growth factors, such as basic fibroblast growth factor (Giardino et al., 1994), which could provide a basis for the slower formation of granulation tissue and wound closure.

Summary of the Invention

The present invention provides a method for treating symptoms of diabetes in a diabetic subject which comprises administering to the subject a therapeutic amount of an agent which inhibits binding of advanced glycation endproducts to any receptor for advanced glycation endproducts so as to treat symptoms of diabetes in the subject.

Brief Description of the Figures

Figure 1. Effect of sRAGE on wound healing in the genetically-diabetic db+/db+ mouse. A full-thickness 1.5 X 1.5 cm wound was created on the backs of db+/db+ mice or control, heterozygote db+/m+mice and covered with TEGADERM®. Diabetic wounds were treated with either phosphate-buffered saline (PBS) directly under the TEGADERM® daily for 7 days commencing on day 3 following surgery or with sRAGE (200 ng). Wound area was measured at baseline through day 21 by placing a glass slide over the wound area, tracing the wound area, and placing this information into a computer in order to calculate the percentage of wound closure as a function of time. Left axis represents percent wound closure.

Figure 2. Administration of sRAGE to the genetically-diabetic db+/db+mouse improves wound healing: dose-response studies. Wounds were created as above and treated from days 3 through 9 with sRAGE (either 2,000, 200, or 20 ng/day) or with phosphate-buffered saline. At day 10, wound area was measured and compared with initial wound area as above. Results are presented as fold increase in percent wound healing compared with mice treated with phosphate buffered saline (defined as one in figure). All statistical analyses are shown comparing wound healing in the presence of different doses of sRAGE vs. treatment of diabetic wounds with phosphate-buffered saline.

Figures 3A and 3B. AGE-immunoreactive epitopes in the wounds of diabetic (db+/db+) mice. 1.5 X 1.5 cm full-thickness wounds created in the backs of diabetic mice(db+/db+ mice; Fig. 3A) and non-diabetic mice (db+/m+;Fig. 3B) were excised, fixed and sections stained with affinity-purified anti-AGE IgG. Magnification: 200X.

Detailed Description of the Invention

The present invention provides a method for treating symptoms of diabetes in a diabetic subject which comprises administering to the subject a therapeutically effective amount of sRAGE so as to treat symptoms of diabetes in the subject. The symptoms may comprise abnormal wound healing, symptoms of a heart attack, symptoms of a stroke, symptoms of peripheral vascular disease, amputation, symptoms of kidney disease, kidney failure, blindness, neuropathy, inflammation or impotence.

The present invention also provides a method for treating symptoms of diabetes in a diabetic subject which comprises administering to the subject a therapeutically effective amount of an agent which inhibits binding of advanced glycation endproducts to any receptor for advanced glycation endproducts so as to treat symptoms of diabetes in the subject. In accordance with the method of this invention, the agent may comprise a polypeptide, a peptidomimetic, an organic molecule, a carbohydrate, a lipid, an antibody or a nucleic acid. In accordance with the method of this invention, the polypeptide may comprise an advanced glycation endproduct polypeptide or a portion thereof, a receptor for an advanced glycation endproduct polypeptide or a portion thereof, a soluble receptor for advanced glycation endproduct polypeptide or a portion thereof. In accordance with the method of this invention, the antibody may comprise an anti-RAGE antibody or an anti-RAGE $F(ab')_2$ fragment. In accordance with the method of this invention, the therapeutically effective amount may comprise a dose of from about 200 ng/day/kg body weight to about 200,000 ng/day/kg body weight or from about 50 ng/day/kg to about 500,000 ng/day/kg body weight.

The present invention provides a method for improving wound healing in a diabetic subject which comprises administering

to the subject a therapeutically effective amount of an agent which inhibits binding of advanced glycation endproducts to a receptor for advanced glycation endproducts, over a sufficient period of time in a sufficient amount so as to improve wound healing in the subject.

In accordance with the method of this invention, the agent may comprise a polypeptide, a peptidomimetic, an organic molecule, a carbohydrate, a lipid, an antibody or a nucleic acid. The polypeptide of this invention may comprise an advanced glycation endproduct polypeptide or a portion thereof, a receptor for advanced glycation endproduct polypeptide or a portion thereof, a soluble receptor for advanced glycation endproduct polypeptide or a portion thereof.

In one embodiment of this invention, the administration may comprise daily administration from about the day of wounding to about ten days after wounding. The present invention provides that the sufficient amount comprises a dose of from about 10 ng/day/kg body weight to about 500,000 ng/day/kg body weight or a dose of from about 150 ng/day/kg body weight to about 200,000 ng/day/kg body weight.

The present invention provides a method for treating symptoms of diabetes in a diabetic subject which comprises administering to the subject a therapeutically effective amount of an agent which agent inhibits binding of advanced glycation endproducts to any receptor for advanced glycation endproducts so as to treat symptoms of diabetes in the subject.

In accordance with the method of this invention, the agent may be a polypeptide, a peptidomimetic, an organic molecule, a carbohydrate, a lipid, an antibody or a nucleic acid. In

the case of polypeptides, the polypeptide may be an advanced glycation endproduct (AGE) polypeptide or a portion thereof, a receptor for advanced glycation endproduct polypeptide or a portion thereof, a soluble receptor for advanced glycation endproduct polypeptide or a portion thereof, e.g., soluble RAGE, or a recombinant polypeptide. The polypeptide may be synthesized chemically or produced by standard recombinant DNA methods. In the case of antibodies, the antibody may be an anti-RAGE antibody or an anti-RAGE $F(ab')_2$ fragment.

In accordance with the method of the present invention, the symptoms which may be treated include abnormal wound healing, symptoms related to having a heart attack, such as chest pain, symptoms related to having a stroke, peripheral vascular disease, amputation, kidney disease, kidney failure, blindness, neuropathy, inflammation and impotence.

The subject on which the method is employed may be any mammal, e.g. a human, mouse, cow, pig, dog, cat, or monkey.

The administration of the agent may be effected by intralesional, intraperitoneal, intramuscular or intravenous injection; by infusion; or may involve liposome-mediated delivery; or topical, nasal, oral, anal, ocular or otic delivery.

In the practice of the method administration may comprise daily, weekly, monthly or hourly administration, the precise frequency being subject to various variables such as age and condition of the subject, amount to be administered, half-life of the agent in the subject, area of the subject to which administration is desired and the like.

In connection with the method of this invention, a therapeutically effective amount of may include dosages which take into account the size and weight of the subject, the age of the subject, the severity of the symptom, the

surface area of the wound, the efficacy of the agent, the method of delivery of the agent and the history of the symptoms in the subject. One of ordinary skill in the art would be readily able to determine the exact dosages and exact times of administration based upon such factors. For example, a therapeutically effective amount may a dose of from about 200 ng/day/kg body weight to about 200,000 ng/day/kg body weight. In this regard, it has been shown that 24 micrograms administered intraperitoneally daily (on days 3-9) to wounded diabetic mice resulted in greatly improved wound healing. In this regard, the dose may also be administered as a single dose or as a series of doses over a period of time.

The present invention also provides a method for improving wound healing in a diabetic subject which comprises administering to the subject a therapeutically effective amount of an agent which inhibits binding of advanced glycation endproducts so as to improve wound healing in the subject.

The present invention provides a method for alleviating inflammation in a subject which comprises administering a therapeutically effective amount of an agent which inhibits binding of advanced glycation endproducts to any receptor for advanced glycation endproducts so as to treat symptoms of inflammation in the subject.

In accordance with the method of the invention, the agent may be a polypeptide, a peptidomimetic, an organic molecule, a carbohydrate, a lipid, an antibody or a nucleic acid. In the case of polypeptides, the polypeptide may be an advanced glycation endproduct polypeptide or a portion thereof, a receptor for advanced glycation endproduct polypeptide or a portion thereof, a soluble receptor for advanced glycation endproduct polypeptide or a portion thereof, or a recombinant polypeptide. The polypeptide may be synthesized

chemically or produced by standard recombinant DNA methods. In the case of antibodies, the antibody may be an anti-RAGE antibody or an anti-RAGE $F(ab')_2$ fragment.

There may be other mechanisms by which soluble RAGE may improve diabetic wound healing. Soluble RAGE may have other effects, such as anti-inflammatory effects that are at least in part, independent of binding up AGE's and interfering with their ability to activate cellular RAGE.

The administration of the agent may be effected by intralesional, intraperitoneal, intramuscular or intravenous injection; by infusion; by liposome-mediated delivery or by topical, nasal, oral, anal, ocular or otic delivery.

In one embodiment of the claimed invention, the administration may include daily administration from about the day of wounding to about ten days after wounding.

In another embodiment of the invention, the sufficient amount may include a dose of from about 200 ng/day/mouse body weight to about 200,000 ng/day/mouse body weight.

The present invention also provides a method for improving wound healing in a diabetic subject which comprises administering to the subject a therapeutic amount of an agent so as to improve wound healing in the subject. The mechanism of improving wound healing may be biochemical in nature or competitive in nature.

As used herein "AGE" means an advanced glycation endproduct; "RAGE" means a receptor for an advanced glycation endproduct; "sRAGE" means a soluble form of a receptor for an advanced glycation endproducts, such as the extracellular two-thirds of the RAGE polypeptide.

In the practice of the methods of the invention a

"therapeutically effective amount" is an amount which is capable of inhibiting the binding of AGE to any receptor for advanced glycation endproduct. Accordingly, the effective amount will vary with the subject being treated, as well as the condition to be treated. For the purposes of this invention, the methods of administration are to include, but are not limited to, administration cutaneously, subcutaneously, intravenously, parenterally, orally, topically, or by aerosol.

Portions of the agent of the invention may be "labeled" by association with a detectable marker substance (e.g., radiolabeled with ^{125}I or biotinylated) to provide reagents useful in detection and quantification of such agent or its receptor bearing cells or its derivatives in solid tissue and fluid samples such as blood, cerebral spinal fluid or urine.

The administration of compounds and pharmaceuticals to subjects to improve wound healing is known in the art because the need for improving the symptoms associated with diabetes has been a long-felt need. The following publications are hereby incorporated by reference: U.S. Patent No. 5,561,116, Solid product containing propolis components, and preparation and uses thereof; U.S. Patent No. 4,971,954, Collagen-based matrices ribose cross-linked; U.S. Patent No. 5,567,417, Method for inhibiting angiogenesis using heparinase; U.S. Patent No. 5,565,428, Method of administration of IGF-1. Administering insulin-like growth factor-I to a mammal so as to sustain its biological activity in the mammal comprising administering a therapeutically effective amount of IGF-I to the mammal for a period of time that stimulates the maximum biological response in the mammal is disclosed. The '428 patent also discloses administration over a period of time and repeated administration and discontinuance of administration for a period as long as necessary to achieve or maintain the

desired biological response in the mammal. Thus, methods of administration of therapeutic amounts of a peptide or protein are known to one of skill in the art. U.S. Patent No. 5,561,137, Thio-heterocyclic macrolactam immunomodulators; U.S. Patent No. 5,561,110, Method for the treatment of the complications and pathology of diabetes; U.S. Patent No. 5,547,672, Accelerated wound healing; U.S. Patent No. 5,532,227, Tetracyclines including non-antimicrobial chemically-modified tetracyclines inhibit excessive glycosylation of different types of collagen and other proteins during diabetes; U.S. Patent No. 5,527,772 Regulation of cell proliferation and differentiation using peptides; U.S. Patent No. 5,468,737, Wound healing accelerated by systemic administration of polysaccharide from aloe; U.S. Patent No. 5,395,398, Microelectric apparatus for the antisepsis, promulgation of healing and analgesia of wound and chronic skin ulcers; U.S. Patent No. 5,378,475, Sustained release drug delivery devices; U.S. Patent No. 5,246,708, Methods for promoting wound healing with deoxyribonucleosides; U.S. Patent No.5,532,227, Tetracycline, including non-antimicrobial chemically-modified tetracycline inhibit excessive glycosylation of different types of collagen and other proteins during diabetes. The disclosures of the publications referred to herein, in their entireties, are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein.

When administered, compounds are often cleared rapidly from the circulation and may therefore elicit relatively short-lived pharmacological activity. Consequently, frequent injections of relatively large doses of bioactive compounds may by required to sustain therapeutic efficacy. Compounds modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl

cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline are known to exhibit substantially longer half-lives in blood following intravenous injection than do the corresponding unmodified compounds (Abuchowski et al., 1981; Newmark et al., 1982; and Katre et al., 1987). Such modifications may also increase the compound's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. As a result, the desired *in vivo* biological activity may be achieved by the administration of such polymer-compound adducts less frequently or in lower doses than with the unmodified compound.

Attachment of polyethylene glycol (PEG) to compounds is particularly useful because PEG has very low toxicity in mammals (Carpenter et al., 1971). For example, a PEG adduct of adenosine deaminase was approved in the United States for use in humans for the treatment of severe combined immunodeficiency syndrome. A second advantage afforded by the conjugation of PEG is that of effectively reducing the immunogenicity and antigenicity of heterologous compounds. For example, a PEG adduct of a human protein might be useful for the treatment of disease in other mammalian species without the risk of triggering a severe immune response. The compound of the present invention capable of improving wound healing in a subject may be delivered in a microencapsulation device so as to reduce or prevent a host immune response against the compound or against cells which may produce the compound. The compound of the present invention may also be delivered microencapsulated in a membrane, such as a liposome.

Polymers such as PEG may be conveniently attached to one or more reactive amino acid residues in a protein such as the alpha-amino group of the amino terminal amino acid, the epsilon amino groups of lysine side chains, the sulfhydryl

groups of cysteine side chains, the carboxyl groups of aspartyl and glutamyl side chains, the alpha-carboxyl group of the carboxy-terminal amino acid, tyrosine side chains, or to activated derivatives of glycosyl chains attached to certain asparagine, serine or threonine residues.

Numerous activated forms of PEG suitable for direct reaction with proteins have been described. Useful PEG reagents for reaction with protein amino groups include active esters of carboxylic acid or carbonate derivatives, particularly those in which the leaving groups are N-hydroxysuccinimide, p-nitrophenol, imidazole or 1-hydroxy-2-nitrobenzene-4-sulfonate. PEG derivatives containing maleimido or haloacetyl groups are useful reagents for the modification of protein free sulfhydryl groups. Likewise, PEG reagents containing amino hydrazine or hydrazide groups are useful for reaction with aldehydes generated by periodate oxidation of carbohydrate groups in proteins.

The invention also provides a kit which comprises a therapeutic amount of an agent, which agent is capable of inhibiting binding of advanced glycation endproducts to a receptor for advanced glycation endproducts, over a sufficient period of time in a sufficient amount so as to treat chronic symptoms of diabetes in the subject. A kit may include a composition which includes sRAGE or a portion thereof in a form which is previously dose regulated and time regulated so that a subject may easily take such therapeutic at home or away from a clinical setting.

This invention is illustrated in the Experimental Details section which follows. These sections are set forth to aid in an understanding of the invention but are not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.

EXPERIMENTAL DETAILS

Example 1: Improved Wound Healing in Diabetic Mice by Treatment with the Soluble Receptor for Advanced Glycation Endproducts (sRAGE)

Defective wound healing in diabetes continues to be an important cause of morbidity in the postoperative period, following trauma, and in the repair of cutaneous lesions. Advanced Glycation Endproducts (AGEs) are the result of nonenzymatic glycation/oxidation of proteins/lipids. Accelerated formation and accumulation of AGEs in tissues of patients with diabetes has been linked, in certain situations, to the development of secondary complications. An important means by which AGEs perturb homeostatic processes is through their interaction with cellular binding sites; the best characterized of these is Receptor for AGE or RAGE, an immunoglobulin superfamily molecule expressed by endothelium, monocytes, and smooth muscle cells, as well as mesangial cells and neurons. AGE engagement of RAGE leads to endothelial activation, with expression of adhesion molecules, enhanced procoagulant properties, and diminished barrier function; and perturbation of monocytes, with changes in cell motility and activation, resulting in expression of proinflammatory cytokines. The interaction of AGEs with RAGE-bearing cells, especially endothelium and mononuclear phagocytes, may promote chronic cellular activation thereby preventing optimal wound healing as reflected by formation of granulation tissue and new connective tissue. The data herein are consistent with this concept: using a secondary intention wound model in diabetic mice, wound closure is enhanced following administration of soluble(s) RAGE, the extracellular domain of the receptor. These experiments contribute to a long-term goal and long-felt need, understanding the contribution of cellular interactions of AGEs in the pathogenesis of diabetic complications.

Poor wound healing in diabetes is likely to be a manifestation of a basic defect in the host inflammatory-reparative response, in addition to possible underlying vascular insufficiency. Exposure of macromolecules to aldose sugars results in nonenzymatic glycation and oxidation (Baynes, 1991; Sell and Monnier, 1989; Ruderman et al., 1992; and Vlassara et al., 1994), initially the reversible early glycation adducts, Schiff bases and Amadori products, form. Following further complex molecular rearrangements, the irreversible AGEs come about. The latter comprise a heterogenous group of structures characterized by fluorescence, propensity to form cross-links, generation of reactive oxygen intermediates (ROIs) and interaction with cellular receptors, the best characterized of which is Receptor for AGE, or RAGE (Schmidt et al., 1992; Neeper et al., 1992; and Schmidt et al., 1994a). AGEs accumulated in the tissues in diabetes influence end-organ function by two general mechanisms: directly, via effects on tissue architecture, consequent to the formation of cross-links and trapping of plasma proteins, and indirectly, by interaction with cellular elements, such as endothelial cells (Ecs), mononuclear phagocytes (Mps), central to homeostasis as well as the host response to pathophysiologically relevant stimuli.

Studies have suggested that the extracellular two-thirds of the molecule, soluble or sRAGE, appeared to be able to inhibit the interaction of circulating AGEs with cellular surfaces (Schmidt et al., 1994b). For example, binding of radiolabelled AGE albumin, a prototypic ligand developed in the laboratory, to cultured endothelial cells or peripheral blood-derived mononuclear phagocytes, was inhibited in the presence of increasing doses of sRAGE. In vivo, clearance of radiolabelled AGE albumin from the circulation of a normal mouse after intravenous injection, was delayed upon treatment with sRAGE. Extrapolation of these findings was

attempted to the setting of wound healing. The goal in these studies was to assess the role of AGE-RAGE interaction in the setting of the host response to wounding.

In order to assess the contribution of AGE-RAGE interaction to defective wound healing in diabetes, the wound healing response in diabetic was compared to normal animals, and to determine if blockade of RAGE would ameliorate wound closure in diabetes. In these studies, it was found that administration of soluble RAGE improved wound healing in genetically-diabetic mice. These data support the hypothesis that RAGE blockade may represent a feasible target for intervention in diabetic wound healing as well as other complications of diabetes, such as renal, retinal, neurological, cardiovascular, cerebrovascular and peripheral vascular diseases. Diabetic subjects experience increased restenosis and local problems after angioplasty which suggests that soluble RAGE may be beneficial in reducing restenosis after balloon/stent injury.

MATERIALS AND METHODS

Murine model of diabetes. A genetic model of insulin-resistant/hyperglycemic diabetes (db+/db+mice) due to an autosomal recessive trait (chromosome 4) which results in abnormalities of glucose metabolism and obesity in homozygote mice was employed. Heterozygote mice (db+/+m) do not develop these abnormalities, and are employed as controls (Coleman, 1982 and Wyse and Dulin, 1970). Diabetic animals are hyperglycemic (glucose>400mg/dl by age 3 months), and develop abnormalities similar to human complications, including a defective wound repair. Life expectancy of homozygote mice is 6-8 months. Wounding studies began when mice reached 8 weeks of age, as AGEs are present by that time.

Model of wound healing. For analysis of wound healing in

diabetes, a secondary intention wound model was employed (Greenhalgh et al., 1990), as it stimulates, in part, the clinical situation following breakdown of skin in an ulcerated area. A full-thickness 1.5 x 1.5 cm wound was created on the back of the mouse which was subsequently covered by TEGADERM (clear, plastic closure). The initial area of the wound was measured by placing a sterile glass slide over the area, and tracing the edges of the wound. The area was then determined by using a computer program (NIH Image 157). Serial measurement of the wound dimensions were made on days 3,5,7,10,14, and 17. This data, consistent with those of previous studies (Greenhalgh et al., 1990), showed significant delay of wound repair in the diabetic mouse especially within the first 2-3 weeks after creation of the wound. Animals in each group were sacrificed at days 17 for analysis. Studies began when mice reached 8-10 weeks of age. In certain experiments, mice were treated with soluble RAGE (the extracellular two-thirds of the molecule) under the TEGADERM on days 3 through 9 after the initial wounding procedure.

Immunohistochemistry for detection of Advanced Glycation Endproducts.

At the time of the wounding procedure, 1.5 X 1.5 cm wounds were excised, fixed in formalin (10%) and then processed for immunohistochemistry using affinity-purified anti-AGE IgG (Miyata et al., 1996).

RESULTS

In order to understand the role of RAGE in diabetic wound healing, 1.5 X 1.5 cm wounds were created on the backs of db+/db+ or db+/m+mice. It was first determined that there was no statistically-significant difference in original wound area among the groups of mice receiving the various treatment regimens. When sRAGE (200 ng/day) was administered under the TEGADERM daily from days 3 through 9, the wound healing observed in diabetic mice was

significantly enhanced compared with diabetic mice treated with vehicle (phosphate buffered saline; $p<0.05$; **Figure 1**). Furthermore, the healing observed in diabetic mice treated with sRAGE approximated that observed in control, db+/m+mice treated with vehicle (differences were not statistically significant). (**Figure 1**).

Consistent with the hypothesis that these findings were due to receptor-mediated mechanisms, dose-response studies revealed that there was no enhancement of diabetic wound healing upon administration of sRAGE, 2,000 ng/day, compared with a daily dose of 200 ng/day (differences were not significant; **Figure 2**). However, consistent with the studies described herein in diabetic mice, treatment with either 200 or 2,000 ng/day sRAGE (administered on days 3 through 9) was significantly superior to treatment of these mice with phosphate buffered saline when the final wound area was measured on day ten after creation of the wound (**Figure 2**). However, at a daily dose of sRAGE of 20 ng/day, there was no significant difference in wound healing in the diabetic mice receiving sRAGE versus those diabetic mice receiving vehicle. (**Figure 2**).

In order to determine if diabetic wounds were enriched in AGE-immunoreactive material, immunohistochemistry was performed of diabetic versus control mice wounds using affinity-purified anti-AGE IgG. These studies demonstrated that there was a significant increase in AGE-reactive material in the wound tissue of the diabetic mice (**Figure 3A**) compared with the nondiabetic control animals (**Figure 3B**).

DISCUSSION

The results of these studies indicate that in diabetic tissue such as wounds, there is increased deposition/formation of AGEs. Such AGEs, upon interaction with their cellular receptor RAGE, result in the generation

of a sustained inflammatory environment in which healing and quiescence of the potent effector cells and mediators is markedly delayed. It was hypothesized that interference with AGE-RAGE interaction might result in accelerated healing. In these studies, it was demonstrated that local administration of soluble RAGE improved diabetic wound healing in a dose-dependent manner. The specific mechanisms which underlie the efficacy of administration of sRAGE is important. It is possible that administration of sRAGE improves any one of a number of important steps in physiologic wound healing such as inflammation, angiogenesis and/or formation and deposition of new granulation tissue, specifically collagen.

Taken together, these data suggest that in an AGE-enriched environment such as that observed in diabetes, interference with AGE-cellular RAGE interaction might result in amelioration of the chronic complications of diabetes. Given that RAGE is expressed in the endothelium and smooth muscle of the vasculature, in mesangial cells, in certain neural and vascular cells of the retina, and in certain neurons of both the central and peripheral nervous systems as well as other cells, it is likely that blockade of cellular RAGE might result in improved diabetic complications that might otherwise lead to heart attacks, stroke, peripheral vascular disease, amputation of the extremities, kidney disease/failure, blindness, impotence and neuropathy. RAGE is found in monocytes and macrophages and may be present in other cell types wherein therapeutic intervention may also be possible. The present studies support the concept that administration of sRAGE (or other forms of RAGE blockade; such as recombinant sRAGE, RAGE-based peptides, anti-RAGE IgG or anti-RAGE $F(ab')_2$) might present a novel form of therapeutic intervention in this chronic, debilitating disorder.

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What is claimed is:

1. A method for treating symptoms of diabetes in a diabetic subject which comprises administering to the subject a therapeutically effective amount of sRAGE so as to treat symptoms of diabetes in the subject.

2. The method of claim 1, wherein the symptoms comprise abnormal wound healing, symptoms of a heart attack, symptoms of a stroke, symptoms of peripheral vascular disease, amputation, symptoms of kidney disease, kidney failure, blindness, neuropathy, inflammation or impotence.

3. A method for treating symptoms of diabetes in a diabetic subject which comprises administering to the subject a therapeutically effective amount of an agent which inhibits binding of advanced glycation endproducts to any receptor for advanced glycation endproducts so as to treat symptoms of diabetes in the subject.

4. The method of claim 3, wherein the agent comprises a polypeptide, a peptidomimetic, an organic molecule, a carbohydrate, a lipid, an antibody or a nucleic acid.

5. The method of claim 4, wherein the polypeptide comprises an advanced glycation endproduct polypeptide or a portion thereof, a receptor for an advanced glycation endproduct polypeptide or a portion thereof, a soluble receptor for advanced glycation endproduct polypeptide or a portion thereof.

6. The method of claim 4, wherein the antibody comprises an anti-RAGE antibody or an anti-RAGE $F(ab')_2$ fragment.

7. The method of claim 3, wherein the symptoms comprise

abnormal wound healing, symptoms of a heart attack, symptoms of a stroke, symptoms of peripheral vascular disease, amputation, symptoms of kidney disease, kidney failure, blindness, neuropathy, inflammation or impotence.

8. The method of claim 3, wherein the subject is a mammal.

9. The method of claim 8, wherein the mammal is a human.

10. The method of claim 3, wherein the administration comprises intralesional, intraperitoneal, intramuscular or intravenous injection; infusion; liposome-mediated delivery; topical, nasal, oral, anal, ocular or otic delivery.

11. The method of claim 3, wherein the agent is administered daily.

12. The method of claim 3, wherein the therapeutically effective amount comprises a dose of from about 200 ng/day/kg body weight to about 200,000 ng/day/kg body weight.

13. A method for improving wound healing in a diabetic subject which comprises administering to the subject a therapeutically effective amount of an agent which inhibits binding of advanced glycation endproducts to a receptor for advanced glycation endproducts, over a sufficient period of time in a sufficient amount so as to improve wound healing in the subject.

14. The method of claim 13, wherein the agent comprises a polypeptide, a peptidomimetic, an organic molecule, a carbohydrate, a lipid, an antibody or a nucleic acid.

15. The method of claim 14, wherein the polypeptide

comprises an advanced glycation endproduct polypeptide or a portion thereof, a receptor for advanced glycation endproduct polypeptide or a portion thereof, a soluble receptor for advanced glycation endproduct polypeptide or a portion thereof.

16. The method of claim 15, wherein the antibody comprises an anti-RAGE antibody or an anti-RAGE $F(ab')_2$ fragment.

17. The method of claim 13, wherein the subject is a mammal.

18. The method of claim 17, wherein the mammal is a human.

19. The method of claim 13, wherein the administration comprises intralesional, intraperitoneal, intramuscular or intravenous injection; infusion; liposome-mediated delivery; topical, nasal, oral, anal, ocular or otic delivery.

20. The method of claim 13, wherein the administration comprises daily administration from about the day of wounding to about ten days after wounding.

21. The method of claim 13, wherein the sufficient amount comprises a dose of from about 10 ng/day/kg body weight to about 500,000 ng/day/kg body weight.

22. The method of claim 13, wherein the sufficient amount comprises a dose of from about 150 ng/day/kg body weight to about 200,000 ng/day/kg body weight.

FIGURE 1

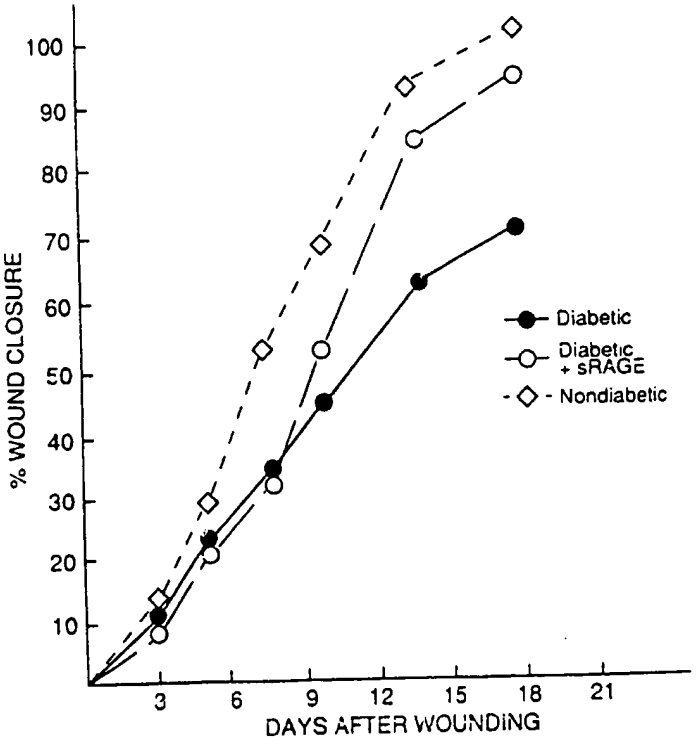

% WOUND CLOSURE
100
90
80
70
60
50
40
30
20
10
3
6
9
12
15
18
21
DAYS AFTER WOUNDING
Diabetic
Diabetic + sRAGE
Nondiabetic

FIGURE 2

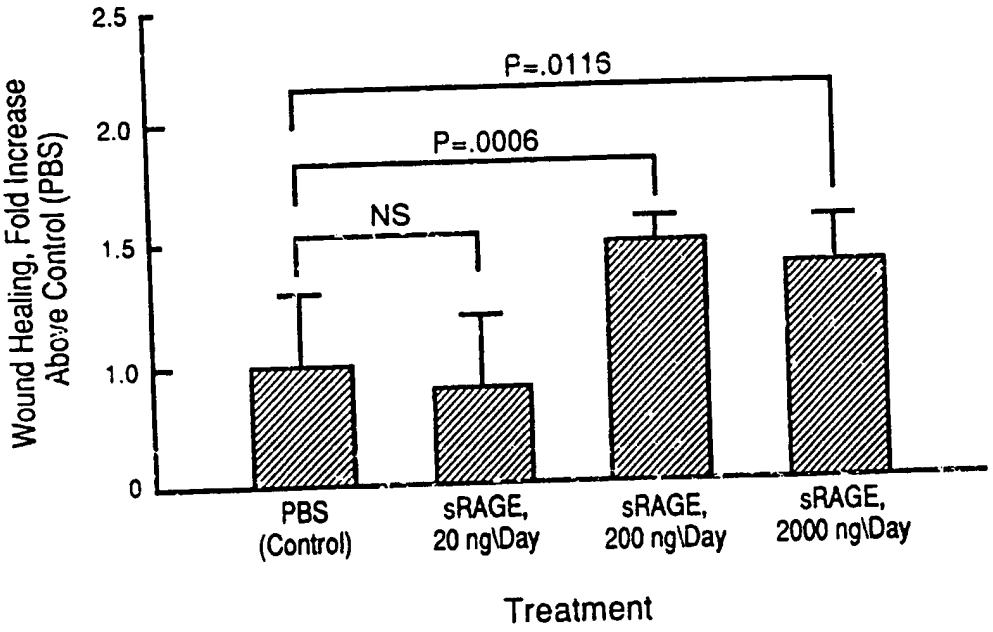
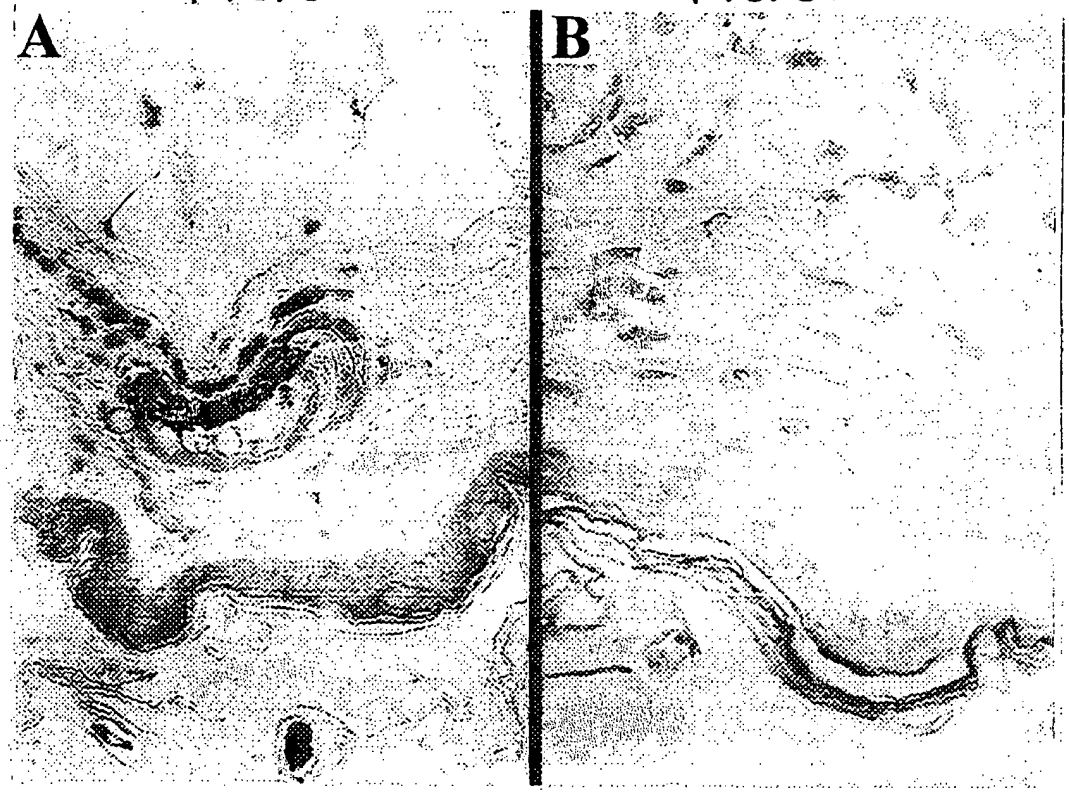

2.5
2.0
1.5
1.0
0
Wound Healing, Fold Increase Above Control (PBS)
P=.0116
P=.0006
NS
PBS (Control)
sRAGE, 20 ng\Day
sRAGE, 200 ng\Day
sRAGE, 2000 ng\Day
Treatment

FIG. 3A

FIG. 3B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/21197

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/395

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1, 152.1. 158.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,436,228 A (POSTLETHWAITE et al.) 25 July 1995, see entire document.	1-22
Y	US 5,561,107 A (JAYNES et al.) 01 October 1996, see entire document.	1-22
Y	SCHMIDT, A.M. et al. Regulation of Human Mononuclear Phagocyte Migration by Cell Surface-Binding Proteins for Advanced Glycation End Products. Journal Of Clinical Investigation. May 1993. Vol. 92, pages 2155-2168, see entire article.	1-22

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
06 JANUARY 1998

Date of mailing of the international search report
09 FEB 1998

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/21197

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	PUGLIESE, G. et al. Upregulation of Mesangial Growth Factor and Extracellular Matrix Synthesis by Advanced Glycation End Products Via a Receptor-Mediated Mechanism. Diabetes, November 1997, Vol. 46, No. 11, pages 1881-1887, especially pages 1884-1885.	1-22
A	US 4,975,421 A (WILLIAMS et al.) 04 December 1990, see entire document.	1-22
A	US 5,165,938 A (KNIGHTON) 24 November 1992, see entire document.	1-22
A	US 5,532,275 (GRUMET) 02 July 1996, see entire document.	1-22
A	US 5,561,110 A (MICHAELIS et al.) 01 October 1996, see entire document.	1-22
A	LI, Y.M. et al. Prevention of cardiovascular and renal pathology of aging by the advanced glycation inhibitor aminoguanidine. Proceedings Of The National Academy Of Sciences, USA. April 1996, Vol. 93, pages 3902-3907, see entire document.	1-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/21197

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/130.1, 152.1. 158.1

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

DIALOG
search terms: advanced glycation endproduct, diabetes, wound healing, vascular disease, renal failure, diabetic, retinopathy, inflammation, impotence